Odor-Induced Persistent Discharge of Mitral Cells in the Mouse Olfactory Bulb

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Matsumoto H, Kashiwadani H, Nagao H, Aiba A, Mori K. Odor-induced persistent discharge of mitral cells in the mouse olfactory bulb. J Neurophysiol 101: 1890–1900, 2009. First published January 21, 2009; doi:10.1152/jn.91019.2008. Short-term retention of sensory information in the form of persistent activity of central neurons plays a key role in transforming a brief sensory stimulation into lasting brain responses. The olfactory system uses this transformation for various functional purposes, but the underlying neuronal mechanisms remain elusive. Here, we recorded odor-evoked, single-unit spike responses of mitral and tufted (M/T) cells in the mouse olfactory bulb (OB) under urethane anesthesia and examined the neuronal mechanisms of the persistent discharge (PD) of M/T cells that outlasts the odor stimulus for tens of seconds. The properties of the persistent afterdischarge that occurred after odor stimulation were distinct from those of odor-induced immediate spike responses in terms of the magnitude, odorant specificity, and odorant concentration–response relationship. This suggests that neuronal mechanisms other than prolonged input from olfactory sensory neurons are involved in generating these afterdischarges. Metabotropic glutamate receptor 1 (mGluR1) is expressed in the dendrites of M/T cells and is thought to participate in intraglomerular interactions among M/T cells. In OBs lacking mGluR1, or treated locally with an mGluR1-selective antagonist, the duration of the odor-induced spike responses was significantly lower than that in control OBs, indicating that mGluR1 within the bulbular neuronal circuits participates in the PD generation. These results suggest that neuronal circuits in the OB can actively prolong the odor-induced spike activity of bulbular output neurons and thus transform a brief odor input into longer-lasting activity in the central olfactory system.

INTRODUCTION

In mammals, a brief olfactory input sometimes causes longer-lasting behavioral, perceptual, and emotional responses. For example, a short exposure to a fox odor, trimethylthiazoline, causes a prolonged fear response in mice that lasts at least a few minutes (Kobayakawa et al. 2007). Although there is evidence that olfactory signals can be prolonged, the neuronal mechanisms that mediate the transition from a brief odor input to the prolonged output responses in the olfactory system are not well understood.

The olfactory bulb (OB) is the first relay in the central olfactory system (Shepherd et al. 2004) and mitral and tufted (M/T) cells in the OB sometimes show a persistent discharge (PD) that outlasts the odorant stimulus for tens of seconds (Lin et al. 2005; Takahashi et al. 2004). In several cortical areas of the mammalian brain, short-term retention of a sensory stimulus is mediated by sustained discharge of cortical neurons in response to a brief sensory stimulus (Fuster 1995; Goldman-Rakic 1995; Major and Tank 2004). Persistent activity of interneurons in Caenorhabditis elegans is considered to be a neuronal correlate of the prolonged behavioral response to odor withdrawal (Chalasani et al. 2007); by analogy, the PD of M/T cells might be responsible for the short-term retention of the odorant stimulus and thus responsible for longer-lasting brain responses.

Odorant-induced PD of M/T cells might be caused by the prolonged activation of the olfactory sensory neurons (OSNs) in the epithelium by remnant stimulus odorants or by the prolonged activation of the signal transduction machinery in OSNs. However, previous studies showed that OSNs cease firing a few seconds after the end of odor application (Chaput 2000; Pirez and Wachowiak 2008; Verhagen et al. 2007). This suggests that neuronal circuits in the OB might have mechanisms for regenerating the activity of M/T cells and that the synaptic inputs from OSNs trigger these mechanisms to generate PD of M/T cells. Here, we addressed whether neuronal circuits in the OB actively participate in the generation of PD and we analyzed in detail the properties of the PD of M/T cells in the dorsal part of the mouse OB.

Olfactory axons synapse on dendrites of M/T cells within the OB glomeruli, which are also the sites of synaptic and nonsynaptic interactions among dendrites of M/T cells and juxtaglomerular neurons (Christie and Westbrook 2006; Kosaka and Kosaka 2005; Schoppa and Westbrook 2001; Shepherd et al. 2004; Wachowiak and Shipley 2006). In the OB slice preparation, M/T cells respond to a single electrical stimulation of olfactory axons with fast excitatory postsynaptic potentials (EPSPs) mediated by α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors and long-lasting slow EPSPs mediated by metabotropic glutamate receptor 1 (mGluR1) and N-methyl-D-aspartate (NMDA) receptors (De Saint Jan and Westbrook 2007). The long-lasting slow EPSP is caused mainly by the secondary release of glutamate from intraglomerular networks and consequent recurrent activation of mGluR1 (De Saint Jan and Westbrook 2007). These results raise the possibility that the recurrent activation of mGluR1 might induce the self-regenerative activity of M/T cells and thereby participate in the generation of PD. In this study, we show that both the duration of the PD and the frequency of encountering M/T cells that show PD are significantly lower in mGluR1-deficient OBs or in OBs treated with an mGluR1 antagonist, thereby raising the possibility that mGluR1 is involved in mediating the PD.

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antagonist. These results underscore the active role of the bulbular neuronal circuits in the generation of PD.

METHODS

Animals

Experiments were performed on 18 adult male C57BL/6 mice (8 to 13 wk old; 20 to 30 g; Japan SLC, Shizuoka, Japan) and three adult male mGlurR1b-rescue mice (C57BL/6 background; 8 to 13 wk old; 20 to 30 g), which were generated by introducing an L7-mGlurR1b transgene expressing mGlurR1b under the control of the cerebellar Purkinje cell–specific L7 promoter (Oberdick et al. 1990) into mGlurR1 knockout mice (Aiba et al. 1994), as described previously (Ichise et al. 2000). mGlurR1 is expressed only in Purkinje cells in mGlurR1b-rescue mice (A. Aiba, unpublished results). Animals were anesthetized with medetomidine (0.5 mg/kg, administered intraperitoneally [ip]) and urethane (0.6 g/kg, ip). Additional doses of urethane were given if necessary. Animals were prepared for acute electro-physiological recording from the OB according to methods described previously (Nagayama et al. 2004). Animals were placed in a stereotaxic apparatus (SR-6N, Narishige, Tokyo). Body temperature was maintained at 37.5°C using a homeothermic heatpad system (MK-900, Muromachi Kikai, Tokyo). Respiratory rhythms were detected using a piezo transducer (MLT 1010, ADInstruments Japan, Nagoya, Japan). All experiments were performed in accordance with the guidelines of the Physiological Society of Japan and the animal experiment committee of the University of Tokyo.

Electrophysiology

For extracellular single-unit recordings from M/T cells, the bone covering the OB and lateral olfactory tract (LOT) was removed using a dental drill and fine forceps. In eight mice, the dura was carefully retracted. A concentric electrode was inserted into the LOT (2.0 mm retracted. A concentric electrode was inserted into the LOT (2.0 mm). In eight mice, the dura was carefully retracted. A concentric electrode was inserted into the LOT (2.0 mm). In eight mice, the dura was carefully retracted. A concentric electrode was inserted into the LOT (2.0 mm).

The interstimulus interval was 3 s. Single-unit pulses (100 μs in duration, 20–100 μA) were used to electrically stimulate the LOT. The interstimulus interval was set to >10 s. The onset and duration (2 s) of odorant delivery were precisely controlled using a custom software program (LabVIEW, National Instruments, Austin, TX). The concentration of odorants at the animal’s nose was 0.3 ppm (n = 2). When 3NH₂ was diluted to 1/10 at the stage of the filter, the concentration of 3NH₂ at the animal’s nose was 0.2 ppm (n = 2).

Statistical analysis

Analyses of spike responses were performed off-line using Spike2 software. The duration of PD was determined as follows: the mean and SD of the firing rate of spontaneous discharges before the odorant stimulation (6 to 1 s before the stimulus onset; bin width = 0.5 s) were calculated. The onset of the spike response was defined as the first bin (0.5 s) in which firing rates exceeded the mean firing rate + 1.5SD of spontaneous discharges. The end of the PD was defined as the first bin to be followed by three successive bins that did not exceed the mean firing rate + 1.5SD of spontaneous discharges. An excitatory immediate spike response (ISR) was defined as a significant increase in spike discharge rate (more than the mean firing rate + 3SD) in bins during the 2 s of odorant presentation. Spike discharges lasting >10 s were defined as PD.
We calculated the normalized magnitude of ISRs and afterdischarges by counting the number of spikes induced by each odorant during the stimulus period (0 to 2 s after the stimulus onset) and poststimulus period (0 to 15 s after the stimulus offset). For the normalization, the number of spikes in each period was divided by the largest number of spikes evoked by the most effective odorant during that period.

To compare the magnitude of spike discharges during ISR and the initial part of the persistent afterdischarge (IP-PAD), the instantaneous and average firing rates during ISR (0 to 2 s after the stimulus onset) and IP-PAD (1 to 3 s after the stimulus offset) were calculated. For this analysis, responses of individual M/T cells to each odorant were examined in at least three trials. The magnitude of spike discharges between ISR and IP-PAD was statistically compared using the two-tailed Mann–Whitney U test. P < 0.05 was considered significant.

For comparing the magnitudes of ISRs (0 to 2 s after the stimulus onset), the firing rate of the largest response was determined for each neuron. The two-tailed Mann–Whitney U test was used to test for significant differences in the magnitude of the largest ISR between M/T cells in wild-type OB and those in mGluR1-deficient OB and between M/T cells in the OB treated with ACSF and those treated with LY367385.

To compare the durations of excitatory spike responses, the duration of the longest excitatory spike response was determined for each neuron. The two-tailed Mann–Whitney U test was used to test for significant differences in the duration of the longest response between M/T cells in wild-type OB and those in mGluR1-deficient OB.

We examined the temporal change of the respiration phase–spike relation of M/T cells as follows. First, we divided the time window of one respiration cycle (360°) into 18 bins (each bin, 20°). For each M/T cell, we calculated the number of spikes occurring in each bin. We then calculated the respiration phase–spike histogram during the four successive respiration cycles after the onset of odor stimulation (during ISR) and determined the bin that showed the peak of the discharge. Next, we calculated the respiration phase–spike histogram during the four successive respiration cycles at a later part of the PAD (3 to 5 s before the end of the PAD). If the peak bin during the ISR differed by more than three bins from that during the later part of the PAD, we defined the M/T cell as showing a temporal change in the respiration phase–spike relation.

RESULTS

Properties of M/T cell PD

To examine the PD properties, we recorded single-unit spike responses of M/T cells in the dorsal OB to a panel of stimulus odorants consisting of homologous series of aliphatic aldehydes (3CHO–7CHO) and alkylamines (3NH2–7NH2). These odorants are known to be effective in inducing PD of M/T cells in the dorsal OB (Takahashi et al. 2004). To ensure the precise timing and duration of odorant stimulation, we used a computer-controlled olfactometer.

Figure 1 contains examples of odorant-induced PDs of M/T cells. The cell in Fig. 1A was strongly activated by the stimulation of the olfactory epithelium for 2 s with 7CHO. The spike response lasted for >30 s, long after the stimulation had ceased. We refer to the PD that occurs after the cessation of odorant stimulation as “persistent afterdischarge” (PAD). Surprisingly, this cell showed such an abrupt increase in firing rate just after the cessation of odorant stimulation that the firing rate at the initial part of the PAD was higher than the firing rate of the ISR that occurred during the stimulation. Then the firing rate of the PAD gradually decreased. We noted that the burst spike discharges during PAD were synchronous with the animal’s respiration cycles (see also Fig. 7). We stimulated this cell eight times with 7CHO and consistently observed PDs with similar durations (39.9 ± 3.1 s, mean ± SD, n = 8) and temporal patterns.

Another cell was strongly activated by heptylamine (7NH2) and the spike discharges lasted for >50 s after the cessation of odorant stimulation (Fig. 1B). In response to 7NH2, this cell consistently showed PDs with durations ranging from 42.0 to 89.5 s (57.5 ± 14.7 s, mean ± SD, n = 10). The burst discharges of this cell during PAD were also synchronous with the respiration cycles. Among 100 M/T cells that showed an excitatory spike response (spike frequency increase during odorant stimulation) to at least one odorant in the stimulus panel, 55 neurons (55.0%) showed PDs >10 s (PAD >8 s).

If the PDs of M/T cells were caused solely by the prolonged activation of OSNs, these burst discharges would be a simple continuation of the ISR of M/T cells that occurs during the period of odorant stimulation. In this case, the magnitude, duration, and odorant specificity of the PAD of an individual M/T cell would be closely correlated with the magnitude and odorant specificity of the ISR of the cell.
To examine this possibility, we first compared the odorant specificity of the PADs and ISRs of individual M/T cells; the responses of a representative cell are shown in Fig. 2. As shown in the peristimulus time histograms (Fig. 2A), this mitral cell showed ISRs to 3CHO–7CHO, with the strongest ISR to 5CHO, and the ISRs gradually decreased with increasing or decreasing carbon-chain length (Fig. 2, A and B). In contrast, PAD was consistently induced by 7CHO stimulation, but not by 5CHO stimulation, which induced only a short-lasting afterdischarge.

In 12 of 22 M/T cells that we stimulated with a homologous series of aliphatic aldehydes or amines, the most effective odorant for inducing PAD was the same as that for inducing ISR. However, in the remaining 10 cells, including the one shown in Fig. 2, the most effective odorant for PAD differed from that for ISR. These discordant responses are inconsistent with the hypothesis that PAD is a simple continuation of the ISR. These results suggest that, at least in the 10 M/T cells with mismatched responses, additional mechanisms other than the prolonged activation of OSNs contribute to PAD.

To further examine whether PAD is a simple continuation of ISR, we next compared the firing rate between the ISR (0 to 2 s after the stimulus onset) and the initial part of the PAD (IP-PAD, 1 to 3 s after the stimulus offset). Both instantaneous and average firing rates of spike responses to 7CHO during the IP-PAD were significantly higher than those of the ISR (Fig. 3A). In other words, the spike-firing rate increased after the cessation of odorant stimulation.

In 9 of 24 M/T cells examined in detail, the firing rate during the IP-PAD significantly exceeded that of the ISR (Fig. 3B), indicating that these M/T cells were activated further after the cessation of odorant stimulation. Thus PAD in these M/T cells is not a simple continuation of the ISR. In the remaining 15 cells, the firing rate during the IP-PAD was not significantly higher than that of the ISR.

We obtained further evidence for differences between ISR and PAD when we examined the odorant concentration–response relationship. Figure 4 shows the changes in the magnitudes of ISR and PAD of a mitral cell induced by odorant stimulation of different concentrations. As the concentration of 5CHO (at the stage of the filter in the olfactometer) increased, this neuron began to show ISR at a concentration of 3/1,000 (3/1,000 [vol/vol] dilution in mineral oil) and a clear ISR at concentrations of 1/100 and 3/100; however, no PAD was induced at these concentrations. With an increase in the concentration to 1/10 and 3/10, this cell showed the largest ISR and showed PAD in some trials, but not at others. Stimulation with the highest concentration (1/1) induced PAD in all the trials, but the magnitude of the ISR was smaller than that induced by stimulation at concentrations of 3/100, 1/10, and 3/10. Thus the odorant concentration–response relationship was clearly different between the ISR and PAD (Fig. 4B). Similar results were obtained in six M/T cells.

These comparisons between the properties of ISR and PAD indicate that PAD is not a simple continuation of ISR, raising the possibility that neuronal mechanisms other than the prolonged activity of OSNs might participate in the generation of odorant-induced PD of M/T cells.

**Involvement of mGluR1 in the generation of PD**

In the isolated OB preparation, a single electrical stimulation of olfactory axons induces fast and long-lasting EPSPs (De Saint Jan and Westbrook 2007; Nowycky et al. 1981). The

**FIG. 2.** In about 45% of M/T cells examined (10 of 22 M/T cells), different odorants were most effective for inducing immediate spike response (ISR) and for inducing PAD. A: responses of a mitral cell to a homologous series of aliphatic aldehydes (3CHO–7CHO). Raster plots and a PSTH of spike responses are shown for each odorant stimulation. This neuron showed ISRs to 3CHO–7CHO and the largest ISR was induced by 5CHO stimulation. In contrast, this cell showed PAD only to 7CHO stimulation. OC at F: 1/10 dilution. B: quantification and comparison of ISR and PAD. The total number of spikes during the stimulus period (0 to 2 s after the stimulus onset, *top panel* [white bars], ISR) and poststimulus period (0 to 15 s after the stimulus offset, *bottom panel* [gray bars]) evoked by stimulation with a homologous series of aliphatic aldehydes was normalized by the maximum total number of spikes during each time window. The most effective odorant for inducing PAD (7CHO) is slightly different in molecular structure from that for inducing ISR (5CHO). Lower and upper error bars indicate the first and third quartile values, respectively.
The filled squares (activation of mGluR1 and NMDA receptors (De Saint Jan and Westbrook 2007). Therefore we hypothesized that the recurrent activation of mGluR1 in the glomerulus might be involved in the generation of PD of M/T cells. To examine this hypothesis, we recorded single-unit activity of M/T cells in the mGluR1-deficient OB of mGluR1b-rescue mice (see METHODS) and compared the odorant-induced PD of M/T cells in mGluR1-deficient OB with that in wild-type OB (Fig. 5). We used the same odorant concentrations for this analysis as in the wild-type mice. In agreement with previous reports (Baude et al. 1993; van den Pol 1995), immunohistochemical examination of OB sections showed that mGluR1 is expressed strongly within the glomeruli and modestly in the external plexiform layer (Fig. 5, A and B). In the mGluR1-deficient OB, we detected no mGluR1 immunoreactivity (Fig. 5B).

An example of an odorant-evoked spike response of an M/T cell in the mGluR1-deficient OB is shown in Fig. 5C. There was no significant difference in the magnitude of ISR between 31 M/T cells in mGluR1-deficient OBs (median magnitude = 25.5 Hz) and 31 M/T cells in wild-type OBs (median magnitude = 26.0 Hz; P = 0.75, two-tailed Mann–Whitney U test; Fig. 5D).

We first noted that the frequency of encountering M/T cells that showed PD was much lower in the mGluR1-deficient OB than that in the wild-type OB. We recorded 31 odorant-responsive M/T cells in mGluR1-deficient OBs and found that only 16.1% (5/31) showed PD (Fig. 5E), whereas 55.0% showed PD in wild-type OBs, as described earlier. In addition, the median duration of the odorant-induced spike responses of M/T cells in mGluR1-deficient OBs (3.0 s) was significantly shorter than the duration in wild-type OBs (14.3 s; P < 0.001, two-tailed Mann–Whitney U test; Fig. 5F). These results suggest that mGluR1 participates in generating the odorant-induced PD of M/T cells.

Since mGluR1 is expressed by a wide variety of neurons in addition to M/T cells in the OB (Baude et al. 1993; Shigemoto et al. 1992), the change in PD of M/T cells in mGluR1-deficient OB might not be due to a lack of mGluR1 in M/T cells. Instead, this change might be induced indirectly by a lack of mGluR1 in other types of neurons, including pyramidal cells in the olfactory cortex (Baude et al. 1993; Shigemoto et al. 1992). To examine whether mGluR1 expressed in the OB is involved in the generation of PD, we locally applied an mGluR1-selective antagonist, LY367385, to the OB surface in wild-type mice. An example of the odorant-evoked spike response of an M/T cell in the OB with LY367385 is shown in Fig. 6A. The magnitude of the ISRs in the OBs treated with LY367385 (median magnitude = 29.8 Hz, n = 34 cells) was not significantly different from that in the OBs treated with ACSF (median magnitude = 29.3 Hz, n = 44 cells; P = 0.86, two-tailed Mann–Whitney U test; Fig. 6B). However, only 8.8% (3/34) showed PD in the OB treated with LY367385, whereas 54.5% of M/T cells (24/44) showed PD in the OB treated with ACSF (Fig. 6C). In addition, the median duration of odorant-induced spike responses of M/T cells in the OB treated with LY367385 (2.5 s) was significantly shorter than that in the OB treated with ACSF (10.8 s; P < 0.0001, two-tailed Mann–Whitney U test; Fig. 6D). These results suggest that the mGluR1 expressed in the neuronal circuits of the OB participates in the generation of PD.

**FIG. 3.** Comparison of the spike discharge rate between the ISR (0 to 2 s after the stimulus onset) and IP-PAD (1 to 3 s after the stimulus offset). **A:** a scattergram comparing the firing rate during ISR and IP-PAD of individual M/T cells induced by the most effective odorant with stimulation. FR, instantaneous firing rate of spike response following the odorant response; IP-PAD, initial part of the persistent afterdischarge. Scale bar: 2 s. **Top inset:** instantaneous firing rate of spike response following the odorant stimulation. FR, firing rate. **B:** a scattergram comparing the firing rate during IP-PAD of individual M/T cells induced by the most effective odorant with that of the ISR induced by the same odorant. Each data point represents an individual M/T cell; x-axis, median magnitude of ISR ([R]ISR); y-axis, median magnitude of the IP-PAD ([R]IP-PAD). The filled squares (n = 9) represent M/T cells that showed a significantly larger mean firing rate during IP-PAD than that during ISR (P < 0.05, 2-tailed Mann–Whitney U test). The filled circles (n = 9) represent M/T cells that showed a significantly larger mean firing rate during ISR than that during IP-PAD. OC at F: 1/10 dilution. Dashed line, x = y. Arrow, [R]IP-PAD > [R]ISR.

(long-lasting EPSP is mediated mainly by the secondary release of glutamate from intraglomerular networks and recurrent activation of mGluR1 and NMDA receptors (De Saint Jan and Westbrook 2007). Therefore we hypothesized that the recurrent activation of mGluR1 in the glomerulus might be involved in the generation of PD of M/T cells. To examine this hypothesis, we recorded single-unit activity of M/T cells in the mGluR1-deficient OB of mGluR1b-rescue mice (see METHODS) and compared the odorant-induced PD of M/T cells in mGluR1-deficient OB with that in wild-type OB (Fig. 5). We used the same odorant concentrations for this analysis as in the wild-type mice. In agreement with previous reports (Baude et al. 1993; van den Pol 1995), immunohistochemical examination of OB sections showed that mGluR1 is expressed strongly within the glomeruli and modestly in the external plexiform layer (Fig. 5, A and B). In the mGluR1-deficient OB, we detected no mGluR1 immunoreactivity (Fig. 5B).

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**Respiration-phase–related modification of PAD**

One candidate mechanism for the generation of PD is the intraglomerular recurrent excitation of M/T cell dendrites via dendritic glutamate release and activation of mGluR1 (De Saint Jan and Westbrook 2007). If the intraglomerular recurrent excitation mechanism that includes mGluR1 and that generates the slow EPSP was not regulated by other neural mechanisms, it would cause continuous tonic spike discharges of M/T cells during the odorant-induced PAD. However, in 83.7% of M/T cells (36 of 43 cells), the spikes during PAD occurred in phase with respiration cycles and were suppressed at specific phases of respiration, raising the possibility that the activity of the excitatory feedback circuit is modulated by the respiration phase. To examine the respiration-phase–related modulation of PAD in detail, we plotted the time course of the relation between respiration phase and spikes (Fig. 7). A representative M/T cell responded to 7CHO with PD that lasted for 63.5 s (Fig. 7A). Without odorant stimulation, this cell tended to fire during the expiration phase. When the nasal epithelium was stimulated with 7CHO, this cell showed intense burst discharges (arrow with * in Fig. 7, B and C) at the early phase of inspiration of the first respiration cycle, followed by spikes in the resting phase. The burst discharges during the inspiration phase decreased in the second respiration cycle and were absent in the third and fourth cycles (Fig. 7B–2). Just after the cessation of odorant stimulation, the spike discharges occurred during the later part of the resting phase and the inspiration phase and then gradually shifted to the inspiration phase and the early part of the expiration phase (Fig. 7C). Such a temporal change in the respiration phase–spike relationship was observed in 66.7% of M/T cells (20/30 cells examined). The temporal change of the respiration phase–spike relationship is inconsistent with the idea that the odorant-induced spike discharges of M/T cells are caused by continued input from OSNs, which are activated during the inspiration phase. The temporal change of the respiration phase–spike relation during PAD rather suggests a gradual change in modulation of the excitatory feedback mechanism by the neuronal circuit in the OB.

**DISCUSSION**

The spike activity of an M/T cell associated with a glomerulus is thought to be shaped mainly by the following factors: 1) excitatory synaptic inputs from OSN axons innervating the glomerulus, 2) self-regenerative activity generated by recurrent excitation in the glomerulus, 3) lateral inhibition through the OB circuit caused by OSN axon inputs to other glomeruli, and 4) lateral inhibition through the OB circuit caused by self-regenerative activity in other glomeruli (Shepherd et al. 2004). Both the ISR and PAD of M/T cells are thought to be shaped by the combination of these four factors, although each of these factors may contribute differentially to ISR and PAD. For example, excitatory synaptic inputs from OSN axons would be quite large during the ISR period, whereas they would be greatly reduced during the PAD period (Chaput 2000; Pirez and Wachowiak 2008; Verhagen et al. 2007). Consequently, the lateral inhibition caused by OSN axon inputs to other glomeruli would be prominent during the ISR period, whereas it would be greatly reduced during the PAD period. This implies that PAD is mainly generated by a combination of self-regenerative activity in the glomerulus and the lateral inhibition caused by self-regenerative activity in other glomeruli.

Our present observations on the disassociation between ISR and PAD are consistent with the above-cited hypothesis. In about 45% of M/T cells, the most effective odorant for inducing ISR was different from that for inducing PAD. In about...
37% of M/T cells, the spike discharge rate during the IP-PAD was significantly larger than that during the ISR. We noted in six M/T cells that the odorant concentration–response relationship was clearly different between the ISR and PAD. During the ISR period, the odorant selectivity, the response magnitude, and the concentration–response relationship of M/T cells would be shaped not only by the OSN axon inputs, but also by the lateral inhibition through the OB circuit caused by OSN axon inputs to other glomeruli. During the PAD period, the possible reduction of the lateral inhibition due to the reduction of OSN axon inputs to other glomeruli would change the odorant specificity, the response magnitude, and the concentration–response relationship. The present results thus suggest that PAD is not a simple continuation of ISR. This notion is also consistent with our observation of the long latency between the end of the ISR and the beginning of the PAD (Figs. 2A and 4A).

The present study does not rule out the possibility that prolonged activation of the OSNs also participates in generating and shaping the PAD. For example, offset responses of OSNs or responses during slow odor clearance might affect the excitatory synaptic input from OSN axons to M/T cells, thus contributing to the shape of the PAD. In addition, mechanical activation of OSNs by breathing (Grosmaître et al. 2007) might also activate M/T cells during the PAD period and the constant OSN input during the PAD period might result in phase-delayed spikes if mitral cell excitability simply decreased during the poststimulus period (Fig. 7). Prolonged activation of OSNs and active prolongation of olfactory axon inputs by the bulbar circuits might work together to transform a brief odor input into longer-lasting activity of M/T cells. However, our results suggest that prolonged OSN activation alone cannot fully explain the characteristics of the PAD that we observed.

$mGluR1$ in the OB participates in the generation of PD

Previous studies in the slice preparation showed that local neuronal circuits within the glomeruli can transform a brief synaptic input from olfactory axons to slow EPSPs in mitral cells that last from hundreds of milliseconds to several seconds (Carlson et al. 2000; Christie and Westbrook 2006; De Saint Jan and Westbrook 2007; Schoppa and Westbrook 2001; Yuan and Knopfel 2006). These studies also showed that secondary release of glutamate from intraglomerular networks and the consequent recurrent activation of M/T cell dendrites via mGluR1 are involved in the generation of slow EPSPs (De Saint Jan and Westbrook 2007; Yuan and Knopfel 2006). Here, we have extended these studies to show that the local neuronal circuit in the OB can actively prolong the odorant-induced spike activity of M/T cells, which lasts for tens of seconds. The local neuronal circuit can thus keep the specific odor information for tens of seconds in the form of PD of specific subsets of M/T cells.

FIG. 5. Metabotropic glutamate receptor 1 ($mGluR1$) is involved in the generation of PD: $A$: immunohistochemical examination of $mGluR1$ expression in the main olfactory bulb (MOB) and olfactory epithelium (OE). Coronal sections of the MOB and OE (from 8-wk-old mice) were labeled with an anti-mGluR1a antibody (orange). mGluR1 was strongly expressed in the MOB, but it was not detectable in the OE. The labeled section was counterstained with DAPI (blue). D, dorsal; V, ventral. Scale bar: 500 μm. $B$: high-magnification images of a labeled section of wild-type OB (WT; 13 wk old) and of mGluR1-deficient OB (KO; 12 wk old). In wild-type OB, the mGluR1 immunoreactivity was observed strongly in all glomeruli and modestly in the EPL, whereas in the mGluR1-deficient OB there was no detectable immunoreactivity. GL, glomerular layer; EPL, external plexiform layer; MCL, mitral cell layer; GCL, granule cell layer. Scale bar: 100 μm. $C$: response of an M/T cell in mGluR1-deficient OB. Raster plots and a PSTH of spike responses to 7CHO stimulation. This neuron invariably showed ISR to 7CHO in each trial. $D$: there was no significant difference in the magnitude of ISR between mGluR1-deficient OB (median ISR, 25.5 Hz [red square; $n = 31$]) and wild-type OB (median ISR, 26.0 Hz [blue square, $n = 31$]; $P = 0.75$, 2-tailed Mann–Whitney $U$ test). Lower and upper error bars indicate the first and third quartile values, respectively. $E$: percentage of M/T cells that showed odorant-induced PD with different durations. A smaller population of M/T cells showed PD ($>10$ s) in mGluR1-deficient OB (16.1% [5/31], red bars) than in wild-type OB (54.8% [17/31], blue bars). These wild-type 31 cells were randomly selected from the 100 odorant-responsive M/T cells. Red columns, M/T cells in mGluR1-deficient OB; blue columns, M/T cells in the wild-type OB; pale columns, M/T cells with odorant-induced responses $<10$ s; dark columns, M/T cells with PD. $F$: the duration of odorant-induced spike discharges (ordinate) in mGluR1-deficient OB (red circles; $n = 31$ cells) was significantly shorter than that in wild-type OB (blue circles; $n = 31$ randomly selected cells; $P < 0.001$, 2-tailed Mann–Whitney $U$ test). Each circle represents the longest duration of odorant-induced spike discharges in an individual M/T cell. The median durations of spike discharges in mGluR1-deficient and wild-type OBs were 3.0 s (red square) and 14.3 s (blue square), respectively. Lower and upper error bars indicate the first and third quartile values, respectively. Gray area indicates spike discharges with duration of $<10$ s. OC at F: 1/10 dilution.
glomerular networks and consequent recurrent activation of mGluR1 in the OB is involved in the generation of PD of M/T cells (Baude et al. 1993; Shigemoto et al. 1992; van den Pol 1995), especially on the square). Lower and upper error bars indicate the first and third quartile values, respectively. C: percentage of M/T cells that showed odorant-induced PD with different durations. Only a small number of M/T cells showed PD in the OBs treated with LY367385 (8.8% [3/34], red bars). In the OBs with ACSF, 54.5% (24/44) showed PD (blue bars). Red columns, M/T cells in the OBs with LY367385 application; blue columns, M/T cells in the OBs with ACSF application; pale columns, M/T cells with odorant-induced responses < 10 s; dark columns, M/T cells with PD. D: the duration of odorant-induced spike discharges (ordinate) in the OBs treated with LY367385 (red circles; n = 34 cells) was significantly shorter than the duration in the OBs treated with ACSF (blue circles; n = 44 cells; P < 0.0001, 2-tailed Mann–Whitney U test). Each circle represents the longest duration of odorant-induced spike discharges in an individual M/T cell. The median duration of spike discharges in the OBs treated with LY367385 was 2.5 s (red square) and in the OBs treated with ACSF was 10.8 s (blue square). Lower and upper error bars indicate the first and third quartile values, respectively. Gray area indicates spike discharges with duration <10 s. OC at F: 1/10 dilution.

M/T cells strongly express mGluR1 (Baude et al. 1993; Shigemoto et al. 1992; van den Pol 1995), especially on the postsynaptic regions of primary dendritic tufts (van den Pol 1995). Our current results with LY367385 indicate that mGluR1 in the OB is involved in the generation of PD of M/T cells. On the basis of these results and previous studies, we speculate that the secondary release of glutamate from intraglomerular networks and consequent recurrent activation of mGluR1 participate in the generation of the odorant-induced PD of M/T cells (Fig. 8).

Whereas the mGluR1-induced slow EPSP lasts for several seconds at most, the PD of M/T cells lasts for tens of seconds. One plausible explanation for the long-lasting activation of M/T cells is the recurrent and mutual excitation between dendritic tufts of M/T cells via glutamate spillover and electrical coupling (Fig. 8; Christie and Westbrook 2006; De Saint Jan and Westbrook 2007; Hayar et al. 2005; Schoppa and Westbrook 2001; Urban and Sakmann 2002). Strong olfactory inputs would result in a large amount of glutamate release from olfactory axon terminals and subsequent secondary release of glutamate from M/T cell dendrites, thereby activating mGluR1 on M/T cell dendrites. The glutamate released from OSN axons might also directly activate mGluR1 on M/T cells. The activation of mGluR1 causes slow EPSPs (De Saint Jan and Westbrook 2007; Yuan and Knopfel 2006) and increases in intracellular Cu2+ concentration via a G-protein–mediated signal transduction cascade (Anwyl 1999; Yuan and Knopfel 2006); therefore M/T cells can release additional glutamate from their dendritic tufts. Long-lasting excitation of individual M/T cells may be coordinated by electrical coupling via gap junctions, which might cause coincident glutamate release from M/T cell ensembles. Thus the recurrent, mutual excitation by mGluR1-mediated glutamate spillover and electrical coupling among M/T cell dendrites within a single glomerulus provides a possible mechanism for the PD.

In vivo two-photon Ca2+ imaging studies have shown long-lasting Ca2+ oscillations phase-locked to respiration cycles in the dendritic tufts of mitral cells, even after the cessation of odorant stimulation (Charpak et al. 2001; Debarbieux et al. 2003), suggesting that an increase in the intracellular Ca2+ concentration due to the activation of mGluR1 in the dendritic tufts of M/T cells might be important for the generation of PAD. Because NMDA receptors have similar effects in prolonging the activity of M/T cells (De Saint Jan and Westbrook 2007), NMDA receptors might also contribute to the increase of intracellular Ca2+ in the dendritic tufts of M/T cells.

In the glomerular layer, there are many types of juxtaglomerular neurons, including excitatory external tufted cells and inhibitory periglomerular cells (Shepherd et al. 2004). External tufted cells associated with the same glomerulus can show synchronized bursting spike discharges and can influence the activity of M/T cells (Hayar et al. 2004, 2005; Urban and Sakmann 2002). GABAergic periglomerular neurons can influence the M/T cell activity via their dendrodendritic synapses within the glomerulus (Rubin and Cleland 2006). Thus the activities of M/T cells are temporally or spatially regulated by these juxtaglomerular neurons (Buonviso et al. 2006; Rubin and Cleland 2006; Wachowiak and Shipley 2006). The present study shows that PAD of M/T cells is phase-locked with respiration cycles and that, during the PAD period, the timing of spike occurrence dynamically shifts in relation to respiration phases (Fig. 7). These results suggest that the recurrent, mutual excitation among M/T cells is modulated by the juxtaglomerular neurons.

Although the glomerular circuits are likely to be the main contributors to the generation of the PD, other bulbar neuronal circuits might also be involved. Because mGluR1 receptors are expressed not only in the glomeruli but also in the external plexiform layer, it is possible that lateral inhibitory circuits formed by dendrodendritic synapses between M/T cells and granule cells might also contribute to the generation of the PD.
Although stimulation with high odorant concentrations typically evokes higher ISRs, it may also elicit greater lateral inhibition and potentially a greater rebound discharge once odorant delivery ceases (Balu and Strowbridge 2007). Odorant stimuli do not disappear instantaneously and one might expect a progressive...
decline in concentration as the odorant clears from the nasal cavity. Rapid decreases in odorant concentration would elicit less mitral cell activity, but the rebound discharge would quickly become more evident and appear as a PD response. Because mGluR1 activation enhances dendrodendritic inhibition (Castro et al. 2007; Dong et al. 2007; Heinbockel et al. 2007), it might contribute to the generation of a greater and longer rebound discharge in some mitral cells and a greater and longer inhibition in other mitral cells. A similar lateral inhibition mechanism at the glomerular level (Augst et al. 2003) might also be involved in the generation of the PD (Fig. 8).

Odorant selectivity of PAD of individual M/T cells

Only a subset of M/T cells that showed ISR also showed PAD. Individual M/T cells responded with a PAD selectively to either one or a few odorants in the panel and, for each M/T cell, the odorant specificity of the PAD was more finely tuned than that of the ISR. The present results also reveal the similarity between odorants that induce PAD and those that induce ISR. In about 55% of the M/T cells that showed PAD, the most effective odorant for inducing PAD was identical to that for inducing ISR. In the remaining M/T cells, the most effective odorant for PAD was not identical, but was similar, to the odorant that produced the largest ISR. These results suggest that the neuronal circuit in the OB can keep the selective odor information for tens of seconds as PAD, even after the cessation of odorant stimulation.

Because in this study we focused on analysis of the properties of PDs, we used a panel of stimulus odorants that are known to be effective in inducing PDs of M/T cells in the dorsal zone of the OB (Takahashi et al. 2004) and did not address under what stimulus conditions the PDs occur. Further analyses with a systematic panel of stimulus odorants and with systematic changes in odorant concentration are necessary to address this problem.

What is the functional significance of PD of M/T cells? M/T cells in the dorsal zone of the OB mediate innate aversive behavioral responses to predator odors and spoiled food odors (Kobayakawa et al. 2007); the odorants in spoiled food include alkyamines and aliphatic aldehydes. This raises the possibility that PD of the dorsal zone M/T cells might be transmitted to higher olfactory centers as a warning signal. In agreement with this idea, we observed that some neurons in the amygdala show a PD in response to alkylamine stimulation that resembles the ISR of M/T cells of the rat olfactory bulb (Chalasani SH, Chronis N, Tsunozaki M, Gray JM, Ramot D, Goodman MB, Bargmann CI. Dissecting a circuit for olfactory behaviour in Caenorhabditis elegans. Nature 450: 63–70, 2007). Although both intrinsic cellular mechanisms and neuronal network mechanisms are thought to be responsible for the generation of persistent activity (Egorov et al. 2002; Major and Tank 2004; Wang 2001), little is known about how glutamatergic synaptic transmission in vivo activates mGluR1 and leads to the mGluR1-mediated PD. Because the mammalian OB has a relatively simple and well-ordered neuronal circuit, it provides an excellent model system to study the mGluR1-mediated intrinsic cellular and neuronal circuit mechanisms for the generation of PD.

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